# FURTHER OBSERVATIONS ON THE SALT REQUIREMENTS OF LIGIA IN BERMUDA [1]

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A previous paper (Barnes, 1932) dealt with the salt requirements and space orientation of the littoral isopod *Ligia baudiniana* in Bermuda. It was shown that *Ligia* is unable to withstand prolonged immersion in sea water and survives best in moist air. The isopod is poikilosmotic and dies within a few hours in distilled water, fresh water, and sea water of half dilution or less. Its survival in natural sea water is not much longer than in artificial sea water even if the latter lacks Mg. Experiments with 5/8 M solutions of single salts established the toxicity series K > Mg > Ca > Na.

The present paper describes the longevity of *Ligia* in weaker solutions of single salts, in antagonistic solutions, and when living in air with access to salt solutions.

## Habits

Although *Ligia* occurs in great numbers on most of the rocky shores of the islands, we have very rarely observed the animal in the act of feeding. Nicholls (1930–31) describes the nocturnal "browsing" of *L. oceanica,* which clings to *Fucus* and cuts off very small portions with its mandibles. During the summer of 1933 it was found that the feeding of *L. baudiniana* can be easily watched if the observer remains motionless on the rocks when the tide begins to recede. The isopods leave their hiding places and follow the ebbing tide, feeding on the algal covering of the damp rocks. Unlike *L. oceanica, L. baudiniana* appears during the day and is positively phototropic in the laboratory. After the intertidal zone is dried by the sun, they usually retreat under stones and in crevices but some remain near the water's edge. The animal scrapes off the unicellular algæ by a vertical motion of the head, the whole anterior half of the body taking part in the rasping movement. The animal feeds for a short interval and moves on for about 10 cm. and feeds again; this intermittent process is especially evident when the intertidal zone is first invaded.

In the preceding paper it was shown that *Ligia* must keep its gills

[1] Contribution from the Bermuda Biological Station for Research.

moist for aerial respiration. Water will rise by capillarity up the spines and uropods or the gills may be brought into contact with a moist substratum by lowering the abdomen. This reflex is elicited by contact with sea water on any part of the body. If a drop of sea water is placed

TABLE I

*Longevity of Ligia in Air and in Water*

| Medium | Year | Average longevity | Maximum longevity | No. specimens tested |
|---|---|---|---|---|
| | | *hours* | *hours* | |
| Distilled water | 1932 | 4 | 5 | 21 |
| | 1933 | 3.9 | 5 | 33 |
| | Both years | 4 | 5 | 54 |
| Fresh water | 1932 | 7½ | 8 | 21 |
| | 1933 | 7 | 9 | 9 |
| | Both years | 7 | 9 | 30 |
| Sea water | 1932 | 34 | 297 | 93 |
| | 1933 | 74 | 240 | 25 |
| | Both years | 42½ | 297 | 118 |
| 2.5 cc. sea water plus 100 cc. $H_2O$ | 1933 | 4½ | 6 | 10 |
| 25% sea water | 1932 | 6 | 10 | 6 |
| | 1933 | 6½ | 7½ | 24 |
| | Both years | 6½ | 10 | 30 |
| 50% sea water | 1932 | 20 | 70 | 7 |
| | 1933 | 17½ | 64 | 15 |
| | Both years | 18 | 70 | 22 |
| Air (dry bowls) | 1932 | 11 | 12 | 8 |
| | 1933 | 17¾ | 25 | 49 |
| | Both years | 15¼ | 25 | 57 |
| Air over damp sand | 1932 | 360 | 625 | 31 |
| | 1933 | 384 | 648 | 51 |
| | Both years | 375 | 648 | 82 |

on the head of an animal which has been out of water some time, the uropods are first brought together and then lowered to the substratum, even if the latter is dry. This reflex is so strong that a running animal drags its spines when traversing a wet region. It is probable that *Ligia baudiniana* is kept moist by its habit of hiding in crevices or under fragments of the porous Bermuda rock which is saturated at high tide. Many specimens are so moist that they glisten in the sun, but I have never found submerged specimens. A pursued isopod will enter the water only if no other path of escape is available but soon returns to air. It is interesting to observe the mass migrations to upper regions of the shore as the tide advances. The individuals at the water's edge are the first to start landwards, but these are joined by specimens several feet from the water line. Individuals of all sizes exhibit this periodic retreat, the mature ones being in advance and always ascending the furthest inland. In captivity the young are released only by females kept in bowls of sea water, but the young (usually about eighty from one specimen) immediately crawl out of the water if any available projection is present. During the past summer the isopods were collected from the intertidal zone at low tide by shaking loose stones over a pail with smooth vertical sides. The specimens dealt with in the previous paper were taken on a wall several feet from the sea. This fact may help to explain differences in the longevity data for the two series (see Table I).

The rôle of positive geotropism as an important factor in the orientation of *Ligia* towards the sea when released some distance inland was confirmed and the tendency of half-grown specimens to crawl down slopes was again observed. In one experiment the isopods crawled toward the sea *up* a slight incline (sand) of 10°, but on a 30° slope in the same region they crawled down *away* from the sea. The orientation of the isopod to shore when released in the sea was studied in tidal pools. Isopods released in the center of a pool swim or crawl to the water's edge in all radial directions regardless of the direction of the mainland. In the water their slow swimming movements make them an easy prey to shore fishes but their rapidity on land enables them to escape pursuit by the mangrove crab *Goniopsis cruentatus*. However, *Ligia* does not occur in mangrove swamps where *Goniopsis* is very abundant.

## Longevity of Ligia in Air and in Water

The number of individuals tested in some of the 1932 experiments was not large and it was necessary to repeat some of the tests, especially since the specimens were taken from a habitat nearer the water line. The same average longevity was found in distilled water,—4 hours,—and in fresh water, 7 hours, but the 1933 isopods lived longer in sea water (Table I). However, we regard the average of the two series, 42 hours, as a satisfactory working average for comparison with other media. The 1933 series of isopods in dry dishes lived an average of 17¾ hours, 6¾ hours longer than the 1932 series. This was probably due to the fact that the 1932 isopods were collected several feet above the high water line and had been exposed to desiccation. In air over damp sand the new series gave a slightly greater longevity, 384 as compared to 360 hours. Moulting was observed only in these specimens and not in submerged ones. The duration of life in 25 per cent and 50 per cent sea water, 6 and 18 hours respectively, was nearly the same in each series. In general it will be seen that the same relative effect was secured in the various media in spite of the difference in year, number of specimens, habitat, and method of collecting. In both series individual specimens were tested in bowls containing 100 cc. of solution.

## Toxicity of Single Salts

The survival of *Ligia* was determined in distilled water containing the percentage of a single salt occurring in sea water for comparison

Table II

*Longevity of Ligia Immersed in Solutions of Single Salts*

| Medium | Average longevity | Maximum longevity | No. specimens |
|---|---|---|---|
| | *hours* | *hours* | |
| 5/8 M NaCl 1933 | 8 | 9 | 18 |
| 5/8 M NaCl 1932 | 8 | 14 | 12 |
| Both years | 8 | 14 | 30 |
| 2.5 cc. 5/8 M NaCl plus 100 cc. $H_2O$ | 2¾ | 3½ | 10 |
| 2.5 cc. 5/8 M $CaCl_2$ plus 100 cc. $H_2O$ | 9¼ | 11 | 30 |
| 2.2 cc. 5/8 M KCl plus 100 cc. $H_2O$ | 3 | 4 | 27 |
| 50 cc. 5/8 M KCl plus 100 cc. $H_2O$ | 2¼ | 2½ | 10 |
| 11.6 cc. 5/8 M $MgCl_2$ plus 100 cc. $H_2O$ | 7 | 9 | 13 |

with the toxicity series of 5/8 M single salts previously described. In a solution containing 2.5 cc. of 5/8 M NaCl plus 100 cc. of water the average duration of life was only 2¾ hours with a maximum of 3½ hours, while in $CaCl_2$ of the same concentration the average longevity was 9¼ hours with a maximum of 11 hours (Table II). The results are of interest since NaCl is the least toxic salt in solutions isosmotic with Bermuda sea water. A 5/8 M NaCl series was repeated and the same average longevity, 8 hours, obtained as previously, showing that the 1933 specimens did not differ in their reaction to sodium chloride. In 2.2 cc. of 5/8 M KCl plus 100 cc. of water, *Ligia* lived an average of 3 hours with a maximum of 4 hours, and durations of approximately the same order were found for 50 cc. 5/8 M KCl plus 100 cc. water, *i.e.*,

TABLE III

*Longevity of Ligia in Antagonistic Solutions*

| Solution | Average longevity | Maximum longevity | No. of specimens tested |
|---|---|---|---|
| | *hours* | *hours* | |
| Artificial sea water (1932) | 40 | 123 | 8 |
| 5/8 M artificial sea water lacking NaCl (1932) | 2 | 2 | 5 |
| Same (1933) | $2\frac{3}{4}$ | $3\frac{1}{2}$ | 12 |
| Artificial sea water lacking NaCl (NaCl replaced by distilled water) | $5\frac{3}{4}$ | 9 | 20 |
| 5/8 M artificial sea water lacking NaCl and $MgCl_2$ | $1\frac{3}{4}$ | $2\frac{1}{2}$ | 10 |
| Artificial sea water lacking Na and Mg (NaCl and $MgCl_2$ replaced by distilled water) | $9\frac{1}{2}$ | 12 | 17 |
| 1 part 5/8 M NaCl plus 1 part 5/8 M $CaCl_2$ | $24\frac{3}{4}$ | 28 | 4 |
| 4 parts 5/8 M NaCl plus 1 part 5/8 M $CaCl_2$ | $51\frac{1}{2}$ | 96 | 15 |
| 2.5 cc. 5/8 M $CaCl_2$ plus 100 cc. 5/8 M NaCl | 21 | 40 | 20 |
| 1 part 5/8 M $CaCl_2$ plus 1 part 5/8 M KCl | 4 | 6 | 10 |
| 2.2 cc. 5/8 M KCl plus 100 cc. 5/8 M NaCl | $10\frac{1}{2}$ | 14 | 10 |
| 4.4 cc. 5/8 M KCl plus 100 cc. 5/8 M NaCl | $6\frac{1}{2}$ | 9 | 10 |
| 11.6 cc. 5/8 M $MgCl_2$ plus 100 cc. 5/8 M NaCl | $9\frac{1}{4}$ | 18 | 20 |

average $2\frac{1}{4}$ hours, maximum $2\frac{1}{2}$ hours. It was reported in the first paper that *Ligia* lives an average of $1\frac{1}{2}$ hours in 5/8 M KCl in which the gills are completely paralysed. In the weak KCl solution, however. the gills vibrated normally but were stopped in the solution containing

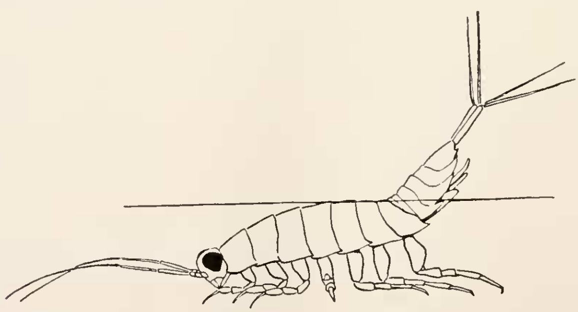

FIG. 1. A specimen in a solution of equal parts NaCl and KCl 5/8 M in which the gill beat is inhibited by the K. The animal has elevated its gills out of the water for aerial respiration.

50 cc. of 5/8 M KCl plus 100 cc. of water. In a solution containing the percentage of Mg in sea water (11.6 cc. of 5/8 M $MgCl_2$ plus 100 cc. of distilled water), the average longevity was 7 hours and the maximum 9 hours. It is clear that the toxicity series in weak solutions is quite different from that in solutions isosmotic with Bermuda sea water.

## Ionic Antagonism

In the first paper it was mentioned that no satisfactory binary mixture was found but antagonism seemed evident between Na and Ca. Further work with salt mixtures supports this statement (*vide* Table III). The most favorable of the binary solutions tested was composed of 80 per cent NaCl and 20 per cent $CaCl_2$ in 5/8 M concentration.

It was of interest to observe that the gills vibrated in the weak KCl solutions, thus showing the toxicity of the potassium ion apart from its inhibition of the gill beat, which is completely inhibited in 5/8 M KCl made in sea water, 5/16 M KCl, and in equal parts of 5/8 M KCl and NaCl. On the other hand, the gill rhythm occurs in the following solutions: 2.2 cc. 5/8 M KCl plus 100 cc. $H_2O$; 2.2 cc. 5/8 M KCl plus 100 cc. 5/8 M NaCl; 4.4 cc. 5/8 M KCl plus 100 cc. 5/8 M NaCl; and in M/4 KCl made in sea water. In solutions inhibiting the gill beat the isopod sometimes elevates its moist gills out of the water for aerial respiration. (Fig. 1.)

Table IV

*Longevity of Ligia in Dry Bowls Containing Watchglasses of Various Solutions*

| Solution in watchglass | Average longevity | Maximum longevity | No. specimens tested |
|---|---|---|---|
| | *hours* | *hours* | |
| Sea water | 292¾ | 432 | 13 |
| Distilled water | 156 | 240 | 19 |
| 25% sea water | 240 | 432 | 8 |
| 1 part 5/8 M NaCl plus 1 part 5/8 M KCl | 10 | 19 | 14 |
| 5/8 M NaCl | 60 | 144 | 16 |
| 5/8 M $CaCl_2$ | 29 | 52 | 22 |
| 2.5 cc. 5/8 M $CaCl_2$ plus 100 cc. $H_2O$ | 156 | 216 | 18 |
| 5/8 M KCl | 8½ | 24 | 24 |
| 2.2 cc. 5/8 M KCl plus 100 cc. $H_2O$ | 168 | 216 | 10 |

## Longevity of Ligia Having Access to Salt Solutions

Watchglasses containing sea water or other solutions were placed in dry bowls containing an isopod (Table IV). In the less toxic solutions the isopod remained at the edge of the watchglass. This was observed with sea water, 5/8 M NaCl, 5/8 M $CaCl_2$, 2.5 cc. of 5/8 M $CaCl_2$ plus 100 cc. of water and 2.2 cc. of 5/8 M KCl plus 100 cc. of water. When the watchglass contained sea water, the isopod crawled through the solution so often that after a few days most of the sea water was in the bowl. In such cases the animal remained in the watchglass if this contained less water than the bowl.

As will be seen in Table IV, the animals lived for long periods with access to sea water, even when diluted to 25 per cent, and to distilled water. It was again possible to demonstrate the toxicity of KCl apart from its paralyzing action on the beating of the pleopods in submerged specimens.

## Discussion

It is of interest to compare the survival of *Ligia baudiniana* in hypotonic solutions with that of *L. oceanica* which appears to be somewhat less terrestrial since it sometimes remains covered by the tide. According to Tait (1916–17) and Nicholls (1930–31), *L. oceanica* survives for very long periods when kept in sea water. Its longevity in diluted sea water is also greater than that of *L. baudiniana,* Tait (1916–17) reporting a survival of as long as forty days in 50 per cent sea water and ten days in 25 per cent sea water (compare with Table I). However, both forms soon succumb in distilled water, the maximum being 5 hours for *L. baudiniana* and 36 for *L. oceanica.* Bateman (1933) finds that the blood of *L. oceanica* is approximately equal in concentration to sea water and that the isopod shows high osmotic independence for a short time when immersed in hypotonic sea water. Animals living on moist sea weed had a salt concentration of 0.631 M, rather greater than sea water. Nicholls (1930–31) kept *L. oceanica* for more than 15 months in moist air with *Fucus.* He reports a more rapid flapping of the pleopods in hypotonic sea water. In one instance 2 liters of sea water containing an isopod were diluted by 25 cc. each day. The time for ten beats decreased from 6 seconds on the fourteenth day to 5 seconds in a month's time. The frequency of gill rhythm of *L. baudiniana* also increases in diluted sea water. The time for ten beats decreased from 3 to 2.7 seconds in a sample specimen when transferred to 50 per cent sea water. Bateman (1933) found that the osmotic pressure of the blood in *oceanica* remained almost normal for several hours in this dilution but after 19 hours the osmoregulation broke down. The increased gill frequency can be interpreted in terms of Schlieper's (1929) theory, as a means of providing the extra oxygen necessary for work performed in the temporary resistance to osmotic forces. The investigations of Fox and Simmonds (1933), for example, show a higher oxygen consumption in species of *Gammarus* inhabiting fresh water.

According to Bogucki (1932), the marine isopod *Mesidotea entomon* exhibits considerable osmoregulation in hypotonic sea water and survives a few days in fresh water and 20 hours in distilled water. It contrasts rather sharply with *Ligia baudiniana,* which has less in common with fresh water animals.

The toxicity series for single salts isosmotic with Bermuda sea water is appreciably different from that for hypotonic solutions of the chief cations. The 5/8 M toxicity series K > Mg > Ca > Na becomes, in the weak solutions, Na > K > Mg > Ca. In the hypotonic solutions permeability is an important factor governing the loss of salts from the blood and Na, the least toxic ion in isosmotic concentration, becomes the most toxic, probably in its action of increasing permeability. In the same way Ca prevents the leaching action of water as Pantin (1931) has described in the estuarine worm *Gunda*. A similar effect has been described by McCutcheon and Lucké (1928) on sea urchin eggs and indeed was shown years ago by Osterhout (1915) in plant cells. *Ligia baudiniana* lives twice as long in 2.5 cc. of 5/8 M Ca plus 100 cc. water as in 2.5 per cent sea water (Tables I and II).

In binary mixtures with Na in natural proportions the Ca solution is the most favorable, but it is interesting to note a greater longevity when the Ca is increased to 20 per cent. Ca, however, does not antagonize the toxicity of K to a great extent (Table III) and in Na-K mixtures the longevity appears to be determined by the amount of K present.

The watchglass experiments showed the same toxicity series for solutions isosmotic with Bermuda sea water as in the immersion tests. With access to weak solutions, however, the isopods exhibited the same longevity with K and Ca. A mixture of equal parts of Na and K showed little antagonism in 5/8 M concentration, the average duration of life being one-thirtieth of that observed when the animals had access to sea water. The amphibious habits of *Ligia* render the isopod particularly suited for the study of specific ion effects which can be determined apart from osmotic and permeability disturbances.

## Summary

1. The toxicity series for the chief cations in sea water for *Ligia baudiniana* changes with the concentration of the single salts. In a weak solution Na is the most toxic but in 5/8 M concentrations it is the least toxic ion.

2. It is suggested that this is due to permeability effects in hypotonic solutions.

3. Na and Ca gave the most favorable binary mixture.

4. When kept in air with access to solutions of single salts *Ligia* shows the same toxicity series with respect to K, Na, and Ca as when immersed in isosmotic solutions.

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[2] For additional references, see Barnes, 1932.